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A study of urinary uric acid to creatinine ratio in assessing the severity of birth asphyxia

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Abstract:
Background:*perinatal asphyxia contributes to almost 20% of neonatal deaths in India*
OBJECTIVE*.To determine prospectively urinary uric acid to creatinine ratio in perinatal asphyxia and showing increased uric acid excretion in early spot urine for identification of perinatal asphyxia.*
Material &Methods*:. The study was conducted in babies admitted to neonatal intensive care unit at niloufer hospital attached to osmania medical college hyderabad on 200 babies delivered between 1/6/2015 to 31/05/2016 Out of which 100 babies are asphyxiated , rest 100 are healthy infants. Urinary uric acid was estimated by auto analyzer by spectrophotometric uricasemethod.urinary creatinine was estimated in same above instrument by using modified kinetic Jaffe's method*
.***Results:*** *Among the 100 neonates in case group, 40 (40%) had increased tone neurologically, 42(42%) had mild and marked hypotonia and 18(18%) were flaccid with severe hypotonia. All the 100 (100%) neonates in control group had normal neurological examination. Abnormal neurological examination is significantly more in cases when compared to Controls with P< 0.001Among the 100 neonates in case group, 40(40%) had no seizures. 60(60%) had seizures as an abnormal neurological examination finding . Abnormal neurological examination is significantly more(60.%) in cases when compared to Controls with P<0.001.Among the 100 neonates in the case group, 40(40%) had mild HIE, 42(42%) had moderate HIE and 18(18%) had severe HIE during the course in NICU. Cases and control were randomly selected, 100 cases (Neonatal Asphyxia) and control of 100 cases (normal newborns), were undertaken in assessing the Urinary Acid / Creatinine ratio as a marker in neonatal Asphyxia, the correlation of of urinary uric acid and creatinine ratio(UUA/Cr) with HIE status among the cases and it was found to be statistically significant with a p value of < 0.001*
Conclusions: *The cut-off UAA/Cr value of >1.4 has 94% sensitivity with a specificity of 96% and has a positive predictive value of 95.52% with a negative predictive value of 94.12%.With an accuracy of 95%*
Keywords: *Perinatal asphyxia, urinary uric acid to creatinine ratio,,apgarscore,HIE*

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I. Introduction

Globally, hypoxia of the newborn (birth asphyxia) or the fetus ("fresh stillbirth") is estimated to account for 23% of the 4 million neonatal deaths and 26% of the 3.2 million stillbirths each year[1]. An estimated 1 million children who survive birth asphyxia live with chronic neurodevelopmental morbidities, including cerebral palsy, mental retardation, epilepsy and learning disabilities. As a result of asphyxia 104 children die every hour[1]. In India, between 250,000 to 350,000 infants die each year due to birth asphyxia, mostly within the first three days of life[2]. Data from National Neonatal Perinatal database (NNPD) suggests that perinatal asphyxia contributes to almost 20% of neonatal deaths in India[2]. In India, 8.4% of inborn babies have a one minute Apgar score less than 7 and 1.4% suffer from hypoxic ischemic encephalopathy (HIE)[2]In India only one third deliveries are institutional[3]and many asphyxiated babies are brought late to hospitals. Perinatal asphyxia may result in adverse effects on all major body systems. Many of these complications are potentially fatal. In a term infant with perinatal asphyxia renal, neurologic, cardiac and lung dysfunction occurs in 50%, 28%, 25% and 23% cases respectively[4]The neuro-developmental delay cannot be assessed with currently used diagnostic methods in patients with neonatal asphyxia or HIE[5,6,7].

Neonatal asphyxia causes neurological morbidity and mortality in full term infants. Despite the increasing understanding of the mechanisms leading to and resulting from neonatal asphyxia, early determination of brain damage following hypoxic-ischemic events still remains the hardest [7,8,9]
In 3 to 13% of infants with cerebral palsy(cp)have evidence of intra partum asphyxia[10]
If there is uninterrupted tissue hypoxia and there is also reperfusion injury, hypoxanthine is oxidized to xanthine and uric acid in presence of xanthine oxidase leading to an increase in uric acid production, which come out in blood from tissues and excreted in urine[11,12,13,14,15].

This study was to evaluate the utility of urinary uric acid to creatinine ratio (UA/Cr ratio) as non-invasive, easy, cheap and at the same time early biochemical means of asphyxia diagnosis and also to find out whether Apgar score is still an important tool for birth asphyxia diagnosis and its severity

AIMS AND OBJECTIVES OF THE STUDY

1)to determine prospectively urinary uric acid to creatinine ratio in perinatal asphyxia and showing increased uric acid excretion in early spot urine for identification of perinatal asphyxia.
2)to compare the ratio of urinary uric acid and creatinine among asphyxiated and non-asphyxiated term neonates admitted in nicu.

II. Materials And Methods

The study was conducted in babies admitted to neonatal intensive care unit at niloufer hospital attached to osmania medical college hyderabad on 200 babies delivered between 1/6/2015 to 31/05/2016 Out of which 100 babies are asphyxiated , rest 100 are healthy infants. Cases and control were randomly selected

Methods of collection of data :

The study included 2 groups:
The case group included 100 cases and 100 controls

Inclusion criteria

Case group included Term Babies admitted to NICU with apgar score of 6 or less at 5 minutes of birth and control group included were apgar score >/=7 at 5 min with no signs of asphyxia.

Exclusion criteria

-Babies with congenital malformations ,suspected metabolic disease on treatment with diuretics ,suffering from anuria and those born to mothers having hypertension ,diabetes mellitus ,toxemia of pregnancy, receiving general anaesthesia, pethidine, phenobarbitone, and other drugs likely to cause depression ,in babies and mother febrile attack within 2 months before delivery were excluded from study.
This study has got the approval from the institutional ethical committee of Osmania Medical College. Informed Consent was obtained from parents/guardians for enrolment in the study.
Collection of sample

- The spot urinary sample within 24 hours is to be collected and is analyzed in hospital laboratory,Urinary uric acid was estimated by auto analyzer by spectrophotometric uricasemethod ,urinary creatinine was estimated in same above instrument by using modified kinetic Jaffe's method.

OBSERVATIONS AND RESULTS

This prospective study was conducted for a period of 12months from june 2015 to May 2016 in Neonatal Intensive Care Unit attached to niloufer Hospital attached to Osmania Medical College hyderabad. Cases and Controls comprised of asphyxiated and non-asphyxiated neonates, respectively.

STUDY DESIGN

A Case study of 100 cases (Neonatal Asphyxia) and control of 100 cases (normal newborns), were undertaken in assessing the Urinary Acid / Creatinine ratio as a marker in neonatal Asphyxia The urine samples from 100 neonates comprising the cases and 100 neonates comprising the controls constituted the material for the study.Among 100 asphyxiated neonates which formed the case group 88(88%) were term gestation, 10(10%) were postdated and 02(02%) were post term. In the control group all neonates were term gestationally .Gestational age distribution is statistically significant with P<0.001Among the 100 neonates in case group, there were 56 (56%) males and 44 (44%) females. Among the control group of 100 neonates, there were 54 (54%) males and 46(46%) females . Samples are gender matched with P=>0.05 insignificant

Table : Place of birth of neonates studied

Place of birth	Cases		Controls	
	No	%	No	%
intramural	74	74.0	100	100.0
Referred from outside	26	26.0	–	–
Total	100	100.0	100	100.0

χ^2 value=29.88 P<0.001 Highly Significant

Mode of delivery of neonates studied

Mode of delivery	Cases		Controls	
	No	%	No	%
Normal	58	58.0	58	58.0
Instrumental	16	16.0	0	0.0
LSCS	26	26.0	42	42.0
Total	100	100.0	100	100.00

χ^2 value=19.764 P<0.001 Highly Significant

Among the 100 neonates in case group, 18 (18%) had Reassuring NST and 58(58%) had Non Reassuring NST suggestive of fetal distress and 24(24%) were referred cases .All the 100 (100%) neonates in control group had Reassuring NST. Incidence of Non Reassuring NST is significantly more in case group (58.0%) against control group with P<0.001. Among the100 neonates in case group, 62 (62%) had Thick MSAF and in 38(38%) the amniotic fluid was clear. All the 100 (100%) neonates in control group clear amniotic fluid. Incidence of thick MSAF was significantly more in cases when compared controls with P<0.001

Apgar score of neonates studied

Apgar score	Cases		Controls		Chi square	P-Value
	No	%	No	%		
At 1 minute						
• <7.0	100	100.0	0	0.0	200	P<0.001 Highly Significant
• >7.0	0	0.0	100	100.0		
At 5 minute						
• <7.0	100	100	0	0.0	200	P<0.001 Highly Significant
• ≥7.0	00	00	100	100.0		

The correlation of APGAR score at 5 min with HIE status amounting the cases and it was found to be statistically significant with p value of < 0.001 there by being helpful as an important tool for birth asphyxia diagnosis and its severity.

Among the 100 neonates in case group, all the 100 (100%) neonates were in need of RESUSCITATION with >1 minute of positive pressure ventilation before stable spontaneous respiration. All the 100 (100%) neonates in control group were not in need of any such intervention and P<0.001 in cases.Among the 100 neonates in case group, 40 (40%) had increased tone neurologically, 42(42%) had mild and marked hypotonia and 18(18%) were flaccid with severe hypotonia. All the 100 (100%) neonates in control group had normal neurological examination. Abnormal neurological examination is significantly more in cases when compared to Controls with P< 0.001

Among the 100 neonates in case group, 40(40%) had no seizures. 60(60%) had seizures as an abnormal neurological examination finding . Abnormal neurological examination is significantly more(60.%) in cases when compared to Controls with P<0.001

Among the 100 neonates in the case group, 40(40%) had mild HIE, 42(42%) had moderate HIE and 18(18%) had severe HIE during the course in NICU.

Out of 100 neonates enrolled in the case group having suffered asphyxia 86(86%) were discharged,06(6%) were discharged against medical advice,08(8%) died.

Comparison of UUA/Cr ratio in two groups studied

UUA/Cr	Cases	Controls
Min-Max	1.3- 4.2	0.52-1.4
Mean ± SD	2.599± 0.820	0.913 ± 0.249
P-value	P<0.001 Highly Significant	

Correlation of clinical variables with HIE status in cases studied

Variables	Total number of patients (n=100)	HIE			χ^{2} VALUE	P VALUE
		Stage I (n=40)	Stage II (n=42)	Stage III (n=18)		
Maternal History						
• Primi	60(60%)	26(43%)	22(37%)	12(20%)	1.765	P>0.05
• Multi	40(40%)	14(35%)	20(50%)	06(15%)		
Gestational age						
• Term	88(88%)	36(41%)	38(43%)	14(16%)	10.99	P<0.05
• Post dated	10(10%)	02(20%)	06(60%)	02(20%)		
• Post term	02(02%)	00(00)	00(00)	2(100%)		
Mode of delivery						
• Normal	58(58%)	26(45%)	24(41%)	08(14%)	4.437	P>0.05
• Instrumental	16(16%)	06(37%)	08(50%)	02(13%)		
• LSCS	26(26%)	08(31%)	10(38%)	08(31%)		
NST						
• Reactive	18(18%)	14(78%)	04(22%)	00(0.0%)	15.89	P<0.001
• Non-reactive	58(58%)	20(34%)	24(42%)	14(24%)		
• Not done	24(24%)	06(25%)	14(58%)	04(17%)		
TMSAF						
• Negative	38(38%)	16(42%)	16(42%)	06(16%)	0.234	P>0.05
• Positive	62(62%)	24(39%)	26(42%)	12(19%)		
Outcome						
• Discharge	86(86%)	40(47%)	42(49%)	04(04%)	74.160	P<0.001
• DAMA	06(06%)	00(0%)	00(0%)	6(100%)		
• Death	08(08%)	00(0%)	00(0%)	8(100%)		

Correlation of urinary uric acid and creatinine ratio(UUA/Cr) with HIE status in cases studied

UAA/Cr	Total number of patients (n=100)				P value
		Stage I (n=40)	Stage II (n=42)	Stage III (n=18)	
Min-Max	1.3-4.2	1.3-2.4	1.6-3.7	3.4-4.2	P<0.001
Mean ± SD	2.599±0.82	1.834±0.339	2.70±0.585	3.866±0.244	

the correlation of of urinary uric acid and creatinine ratio(UUA/Cr) with HIE status among the cases and it was found to be statistically significant with a p value of < 0.001

Shows sensitivity, specificity and predictive values of UAA/Cr in prediction of Neo-natal asphyxia

UAA/Cr	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
>1.4	94.00	96.00	95.92	94.12	95	<0.001

PPV- Positive predictive value, NPV- Negative predictive value

III. Discussion

The value of present biochemical markers for diagnosing asphyxia is inadequate and controversial[16.]The Apgar score has a limited role in predicting the immediate outcome,such as that of HIE and the long-term sequelae[18.]Several studies have shown that cerebral function monitoring using non invasive techniques, such as EEG within six hours of birth, cranial ultrasonography, cranial topography, doppler measurements of cerebral blood flow, somato-sensory evoked potentials, magnetic resonance imaging and estimation of neurophysiological markers such As CK-BB, brain specific LDH isomer, glutamate and neurone specific enolase in the cerebrospinal fluid are all useful in predicting both the immediate dysfunction and the long term outcome[17,19,20]. In the present study an attempt has been made to ascertain whether urinary uric acid and creatinine ratio(UUA/Cr) can distinguish an asphyxiated from a non-asphyxiated term neonate. These tests are routinely available in most centers and hence a comparative study was done to establish the usefulness based on previous studies an attempt was also made to find out whether Apgar score is still an important tool for birth asphyxia diagnosis and its severity

Because urinary creatinine can be used as the reference substrate in a spot urine sample, an increased UA/Cr ratio (as a sign of increased ATP degradation) may be a valuable indicator of the severity of tissue hypoxia in patients with intact renal functions[21].The present study revealed significant increase in UA/Cr ratio in early spot urine samples from asphyxiated full term newborns and the study proved positive correlation between the urinary UA/Cr ratio and the severity (grading) of HIE (P< 0.001). In the study the two infants with the highest UA /Cr ratio died in the early neonatal period.

Study found sex of the baby and birth weight of the neonate not to be statistically significant difference between the cases and control groups. But mode of delivery was found to be statistically significant in both studies with the cases group having statistically significant more number of instrumental deliveries as well as cesarean sections. Studies APGAR SCORE at 1 minute ,5 minute,10 minute to statistically significant between the case and the control group there by being helpful as an important tool for birth asphyxia diagnosis and its severity.

. Comparative study of baseline characteristics of cases and controls.between our study an as reported by Reem Mahmoud and Dina El Abd[22]

Characteristics		Reem Mahmoud and Dina El Abd		Present study		P value
		Cases (n=40)	Con trols (n=20)	Cases (n=100)	Controls (n=100)	
sex	Male	40%	70%	56%	54%	
	Female	60%	30%	44%	46%	
Mode of delivery	Normal	70%	80%	58%	58%	
	instrumental			16%	0%	
	Cesarean	30%	20%	26%	42%	
Birth weight		3.25±0.543	3.32±0.442	2.733± 0.346	2.812± 0.329	
APGAR	1min 5 min	1(0-1) 3(1-5)	9(8-10) 9(9-10)			
Urinary uric acid and creatinine ratio		2.9 ±0.73	0.72±0.35	1.3- 4.2	0.52-1.4	

In the above study a significant correlation was detected between the UA/Cr ratio and the severity of HIE in the asphyxiated group (P< 0.01) similar to what we have reported as shown in table

Table : The urinary uric acid / creatinine ratio in relation to HIE reported byReem Mahmoud and Dina El Abd[22]

	Mild HIE	Moderate HIE	Severe HIE	Controls
Number	16	16	8	20
UA/Cr	1.53 ±0.25	2.19±0.32	3.I8 ±0.6 1	0.72±0.35
Range	1.02-2.11	1.68-2.69	2.25- 4.54	0.20-1.22

Asphyxiated neonates classified into 3 groups as per sarnat and sarnat staging 1976 similar to that used in our study

Table : Correlation of UAA/Cr with HIE status in my case

UAA/Cr	Total number of patients (n=100)	HIE			P value
		Stage I (n=40)	Stage II (n=42)	Stage III (n=18)	
Min-Max	1.3-4.2	1.3-2.4	1.6-3.7	3.4-4.2	<0.01
Mean ± SD	2.599±0.82	1.834±0.339	2.70±0.585	3.866±0.244	

The 2 infants who died with severe HIE had the highest UA/Cr ratios (4.54, and 4.50) as reported by the above study which is similar to our study also in which the highest UA/Cr ratios (4.1,4.2) who were classified into HIE stage 3 expired.In above study by PallabBasu et al(2008) [23]it was found that urinary UA/Cr ratio was significantly higher in cases than controls (3.1± 1.3 vs 0.96 ± 0.54; $p < 0.001$) which is similar to my study asshown in. It was also found that there was significant difference between Apgar scores of cases than controls (3.8± 1.4 vs 9.60± 0.38; $p = 0.02$). Mean urinary UA/Cr ratio for Apgar score 4 – 6 were 1.97±0.32 vs 0.96 ± 0.54; $p < 0.003$(cases vs. controls) and Apgar score 0 – 3 were 4.24±0.81 vs 0.96 ± 0.54; $p < 0.001$ (cases vs. controls). There werealsosignificant differences of mean urinary UA/Cr ratio for Apgar score 4 – 6 vs Apgar score 0 – 3 ($p < 0.001$) which is also similar 2 our study
where apgar at 1 min,5 min and 10 min were found to be useful in diagnosis of asphyxia and its severity.

Comparative study of results of our study an as reported by BADER et al[24]

	UAA/Cr cutoff	Sensitivity	Specificity	PPV	NPA	P value
PRESENT STUDY	>1.4	94%	96%	95.9%	94.1%	<0.001
BADER et al	>1.2	74%	76%	78%	72%	<0.001**

The positive predictive value of Ua/Cr >1.2 was 78% and the negative predictive value was 72%.The sensitivity was 74% and specifity 76%.which was similar to our study , difference being we had taken the cut off value of Ua/Cr>1.14.

My results support those of Chen et al(1999) [25]who suggested that urinary ratio of UA to creatinine was significantly higher in both full term and preterm infants with perinatal asphyxia than in those without PA. Banupriya et al(2008) [26]reported that urinary excretion rate of uric acid in addition to malondialdehyde and proteins are significantly higher in PA and correlates with APGAR and the severity of HIE. This coincided with my results in which hypoxic newborns with UA/Cr ratio of >4.1. [26].The results of the present study were in concordance with those of Akisu et al(2007) [27]who reported elevated urinary UA/ CR ratio in full term infants with PA and that the ratio correlates with the severity of HIE.

However, we found the UA/Cr ratio to be a good, simple screening test for the early assessment of perinatal asphyxia, Furthermore, there is a correlation between the UA/Cr ratio and the severity of the encephalopathy, indicating the degree of injury at an early stage when other quantitative methods frequently cannot be carried out.

IV. Conclusion

- Large amounts of oxygen radicals that are produced in the reoxygenation period, following asphyxia and it is expected that high levels of uric acid are produced and excreted in the early part of first day of life. Because urinary creatinine can be used as the reference substrate in a spot urine sample, an increased UA/Cr ratio (as a sign of increased ATP degradation) may be a valuable indicator of the severity of tissue

hypoxia in patients with intact renal functions. UA/Cr ratio estimation from spot urine sample is available in most centres and are comparatively cheaper tests.

- The cut-off UAA/Cr value of >1.4 has 94% sensitivity with a specificity of 96% and has a positive predictive value of 95.52% with a negative predictive value of 94.12%.Withan accuracy of 95%
- So urinary uric acid to creatinine ratio can be used as an additional non-invasive ,easy and at the same time early biochemical marker of birth asphyxia which biochemically supports the clinical diagnosis and severity grading of asphyxia by apgar score.

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